Original Article

Endogenous ApoE Expression Modulates Adipocyte Triglyceride Content and Turnover

Zhi Hua Huang,[1] Catherine A. Reardon,[2] and Theodore Mazzone[3]

Apolipoprotein E (apoE) is highly expressed in adipose tissue and adipocytes in which its expression is regulated by peroxisome proliferator–activated receptor (PPAR)-γ agonists and tumor necrosis factor–α. There is, however, no information regarding a role for endogenous apoE in differentiated adipocyte function. In this report, we define a novel role for apoE in modulating adipocyte lipid metabolism. ApoE$^{-/-}$ mice have less body fat and smaller adipocytes compared with wild-type controls. Freshly isolated adipose tissue from apoE$^{-/-}$ mice contains lower levels of triglyceride and free fatty acid, and these differences are maintained in cultured adipocytes derived from preadipocytes. Adenoviral expression of apoE in apoE$^{-/-}$-cultured adipocytes increases triglyceride and fatty acid content. During incubation with apoE-containing triglyceride-rich lipoproteins, apoE$^{-/-}$ adipose tissue accumulates less triglyceride than wild type. The absence of apoE expression in primary cultured adipocytes also leads to changes in the expression of genes involved in the metabolism/turnover of fatty acids and the triglyceride droplet. Markers of adipocyte differentiation were lower in freshly isolated and cultured apoE$^{-/-}$ adipocytes. Importantly, PPAR-γ–mediated changes in lipid content and gene expression are markedly altered in cultured apoE$^{-/-}$ adipocytes. These results establish a novel role for endogenous apoE in adipocyte lipid metabolism and have implications for constructing an integrated model of adipocyte physiology in health and disease. *Diabetes* 55:3394–3402, 2006

Obesity and its consequent insulin resistance are major health problems in the U.S., imparting significant risk for diabetes and cardiovascular disease (1–4). The prevalence of obesity is predicted to substantially increase over the next several decades, and there is a need to better understand adipocyte and adipose tissue physiology. In the past several years, it has become apparent that adipocytes and adipose tissue actively modulate systemic substrate availability and produce a number of protein factors with endocrine, paracrine, and autocrine regulatory activity (5,6). Apolipoprotein E (apoE), which was first described as a product of hepatocytes and a surface component of lipoproteins, e.g., in humans, chylomicrons, VLDL, remnant lipoproteins, and HDL, has been shown to be highly expressed in adipocytes and adipose tissue (7). Interestingly, apoE has been shown to be highly expressed in a number of cell types that experience high lipid flux (8–15). The physiologic role of apoE expression in other cell types has been intensively studied and characterized (8–15). In macrophages and steroidogenic cells, for example, endogenous apoE expression plays an important role in cellular lipid balance. Adipocytes and adipose tissue, like macrophages and steroidogenic cells, also experience large lipid fluxes integral to their differentiated function, yet there is no information regarding a potential physiologic role of apoE expressed in the adipocyte. Our laboratory has recently reported that adipocyte and adipose tissue apoE expression is modulated by tumor necrosis factor–α and peroxisome proliferator–activated receptor (PPAR)-γ agonists in vitro and in vivo (16). These two factors are also important for regulating the expression of a number of adipocyte genes with established roles in modulating adipocyte substrate turnover and metabolism (17–19). In view of the above considerations, we investigated a role for endogenous adipocyte apoE in modulating adipocyte differentiated function. Our results establish a novel role for endogenous adipocyte apoE in regulating adipocyte lipid content and metabolism.

RESEARCH DESIGN AND METHODS

Cell culture medium and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Chemicals were from Sigma (St. Louis, MO), and organic solvents were from Fisher Scientific (Pittsburgh, PA). [^{3}H]acetate and [^{14}C]oleate were obtained from Perkin-Elmer (Wellesley, MA), rosiglitazone was purchased from Biomol (Plymouth Meeting, PA), and triglyceride, free cholesterol, and free fatty acid assay kits were from Wako Chemicals USA (Richmond, VA). Adipocyte differentiation medium and maintenance medium were from Zen Bio (Research Triangle Park, NC).

Adipocyte isolation and culture. ApoE$^{-/-}$ mice or wild-type controls were purchased from The Jackson Laboratories (Bar Harbor, ME). All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Illinois, Chicago. For experiments, inguinal fat pads (IFPs) were collected from age-matched (10–12 weeks) and sex-matched apoE$^{-/-}$ or wild-type mice. Preadipocytes were isolated by digesting freshly isolated adipose tissue with 0.5 mg/ml collagenase in Dulbecco's modified Eagle's medium (DMEM) for 1 h at 37°C in a shaking water bath. Stromal-vascular cells (containing preadipocytes) were spun down and washed twice with DMEM and filtered through a polypropylene mesh (pore size 150 μm). After centrifugation, floating cells (freshly isolated adipocytes) and the cell pellet (stromal-vascular cells) were used for RNA isolation. Other cell pellets were resuspended in DMEM with 10% FBS and cultured in T75 flasks for 2–3 days before passaging. Cells were passaged once before being seeded into a six-well plate for experiments. Preadipocytes were differentiated into adipo-

From the [1]Department of Medicine, University of Illinois, Chicago, Illinois; the [2]Department of Pathology, University of Chicago, Chicago, Illinois; and the [3]Departments of Medicine and Pharmacology, University of Illinois at Chicago, Chicago, Illinois.

Address correspondence and reprint requests to Theodore Mazzone, MD, Section of Endocrinology, Diabetes and Metabolism (MC 797), University of Illinois, 1819 W. Polk St., Chicago, IL 60612. E-mail: tmazzone@uic.edu.

Received for publication 17 March 2006 and accepted in revised form 23 August 2006.

apo, apolipoprotein; CEBP, CCAAT/enhancer binding protein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; IFP, inguinal fat pad; PID, post-induction day; PPAR, peroxisome proliferator–activated receptor; TGRL, triglyceride-rich lipoprotein.

DOI: 10.2337/db06-0354

TABLE 1
PCR primer sets

Gene name	Forward primer	Reverse primer
ACADM	CAAATGCCTGTGATTCTTGCT	CGTCACCCTTCTTCTCTGCTT
PGC-1α	ACTACAGACACCGCACACACC	CCTTTCGTGCTCATAGGCTTC
Adiponectin	GGAGATGCAGGTCTTCTTGGT	TCTCCAGGCTCTCCTTTCCT
Perilipin	GAAGCATCGAGAAGGTGGTAGA	GCATGGTGTGTCGAGAAAGAG
Caveolin-1	GGCAACATCTACAAGCCCAAC	GTCGAAACTGTGTGTCCCTTC
β-Actin	CTGGGACGACATGGAGAAGA	AGAGGCATACAGGGACAGCA
PPAR-γ	GCCCTTTGGTGACTTTATGGA	GCAGCAGGTTGTCTTGGATG
ACO	CTATGGGATCAGCCAGAAAGG	AGTCAAAGGCATCCACCAAAG
CPT-1	CTAACCTCCAACCACCGAAAC	TTGAACTTGCTACCACCACCA
CEBPα	TGTTGGGGATTTGAGTCTGTG	GGAAACCTGGCCTGTTGTAAG
FAS	ATTCGGTGTATCCTGCTGTCC	TTGGGCTTGTCCTGCTCTAAC

ACADM, acetyl-CoA dehydrogenase, medium chain; ACO, acyl-CoA oxidase; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase; PGC-1, PPAR-γ coactivator.

cytes by addition of adipocyte differentiation medium containing 10% FBS, rosiglitazone, insulin, dexamethasone, and isobutylmethylxanthine for 3 days. The end of this 3-day incubation is designated as day 0. Cells were then incubated with adipocyte maintenance medium containing 10% FBS, insulin, and dexamethasone for the times indicated in the figure or table legends. All experiments using cultured adipocytes refer to adipocytes derived from preadipocytes differentiated in culture using the above-mentioned differentiation medium.

To express apoE in isolated apoE$^{-/-}$ adipocytes, a human apoE3 adenovirus was incubated with cell monolayers at a multiplicity of infection of 100 for 4 h in DMEM. Control cultures were incubated with a LacZ adenovirus. After 4 h, cells were washed and placed in DMEM with 10% FBS for the times indicated in the figures. Adenovirus expression of apoE was confirmed by Western blotting of cell monolayers and culture medium.

Adipocyte sizing and quantitation of total body fat mass. Inguinal fat from wild-type or apoE$^{-/-}$ mice was rinsed in PBS and fixed in 4% paraformaldehyde in PBS with 5% sucrose overnight at 4°C. Paraffin-embedded samples were sliced (10-μm thickness) and stained with hematoxylin-eosin. The diameter of adipocytes was estimated using Open Lab Software (version 1.5). For each mouse genotype, a minimum of 100 cells was measured. To measure total body fat mass, mice were scanned with a *p*-Dexa Scanner (Norland/Stratec, Pforzheim, Germany). Total body fat mass was analyzed with Saber Research Software (Norland/Stratec).

Adipose tissue organ culture. Freshly isolated IFPs were washed in PBS and placed in 60-mm dishes at 100 mg total tissue (wet weight) per dish. The fresh tissue was minced into 1-mm pieces and incubated in DMEM with 1% lipoprotein-deficient FBS with or without 100 μg/ml apoE-containing VLDL for 48 h. ApoE-containing $d < 1.006$ g/l lipoproteins were isolated by density gradient ultracentrifugation of nonfasting human plasma, after a spin at 26,000*g* at 10°C for 30 min to remove chylomicrons, as previously described (20). The layer containing buoyant triglyceride-rich lipoproteins (TGRLs) was collected and dialyzed against PBS at 4°C for 24 h. Pre-β mobility of the isolated fraction was confirmed using a Titan Lipoprotein Gel System (Helena Laboratories, Beaumont, TX), and apoE content in the TGRL fraction was confirmed by Western blotting.

Lipid assays. For measurement of triglyceride synthesis, cells were washed, incubated in serum-free medium for 30 min, and then pulse-labeled with 0.25 μCi/ml [^{14}C]oleate/BSA complex (specific activity 70,000 dpm/nmol) in DMEM for 2 h. After washing, cell lipids were extracted in hexane:isopropanol (3:2), and triglyceride was separated by thin layer chromatography in a solvent system of hexane:ethyl ether:acetic acid (90:30:1). Triglyceride spots were scraped into scintillation fluid for analysis. Triglyceride hydrolysis rate was estimated by measuring glycerol released into the medium over 2 h using a Free Glycerol Determination kit (Sigma). To measure free cholesterol synthesis, cells were pulse labeled with 50 μCi/ml [^{3}H]acetate in DMEM with 0.2% BSA for 4 h. Cells were washed, lipids were extracted with hexane/isopropanol, and free cholesterol was separated by thin layer chromatography as described above. Triglyceride, free fatty acid, and free cholesterol mass was measured in hexane/isopropanol cell extracts using enzymatic assay kits from Wako Chemicals USA.

mRNA quantitation. Total RNA was extracted using a Qiagen kit (Valencia, CA). First-strand cDNA was synthesized from 1 mg total RNA using random hexamer primers according to the manufacturer's instructions (Fermentas, Hanover, MD). Real-time PCR was performed on each sample in triplicate using the Mx3000p Quantitative PCR System (Stratagene, La Jolla, CA). Reactions were carried out in a total volume of 25 μl using Brilliant SYBR Green QRT-PCR Master Mix (Stratagene). Relative quantitation for each gene (expressed as fold increase over control) was calculated after normalization to β-actin RNA. Primer pairs used for each gene are shown in Table 1.

Other assays. Cell protein was measured with a DC Protein kit (Bio-Rad, Hercules, CA). Western blotting was performed as previously described in detail (16). DNA was extracted from cell/tissue using DNAzol (Invitrogen), and its mass was estimated using spectrophotometry.

Statistical analysis. Each experiment shown is representative of three to five experiments (each done in triplicate using at least six mice of each genotype) with similar results. Statistical significance of observed differences were analyzed using Student's *t* test or ANOVA using SPSS (Chicago, IL).

RESULTS

Adipocyte size and fat mass in apoE$^{-/-}$ mice. We compared adipocyte diameter and adipose tissue mass in apoE$^{-/-}$ or wild-type controls maintained on a chow diet. The results shown in Fig. 1*A* are representative of three separate comparisons and show that adipocytes were smaller in white adipose tissue harvested from apoE$^{-/-}$ mice. The results in Fig. 1*B* show that IFP mass represented a smaller percentage of total body weight in apoE$^{-/-}$ mice. Results in Fig. 1*C* confirmed that total body fat mass, as measured by dexa scanning, represented a lower percentage of total body weight in apoE$^{-/-}$ mice compared with wild-type controls. The above results indicate that the systemic absence of apoE in chow-fed mice leads to less adipose tissue and smaller adipocytes.

Evaluation of endogenous adipocyte apoE expression on lipid content and metabolism in adipocytes. To evaluate a physiologic role for endogenous adipocyte apoE in adipocyte lipid metabolism, we compared the triglyceride and free fatty acid mass in freshly isolated adipose tissue and in isolated adipocytes (derived from preadipocytes) obtained from apoE$^{-/-}$ and wild-type control mice. We first confirmed, in separate experiments, that 80 ± 3% of total apoE mRNAs in freshly isolated adipose tissue was present in the suspended adipocyte fraction of a collagenase digest, with the balance in the stromal-vascular fraction. Figure 2*A* shows that triglyceride and free fatty acid content is significantly lower in freshly isolated adipose tissue from apoE$^{-/-}$ mice compared with wild-type control, consistent with the adipocyte size data shown in Fig. 1*A*. Figure 2*B* shows that the decrease in triglyceride and free fatty acid mass is also present when comparing adipocytes isolated from apoE$^{-/-}$ mice with those isolated from wild-type control mice, even after ex vivo culture and differentiation from preadipocytes. The reduction in triglyceride per milligram protein in cultured adipocytes from apoE$^{-/-}$ mice was accompanied by a

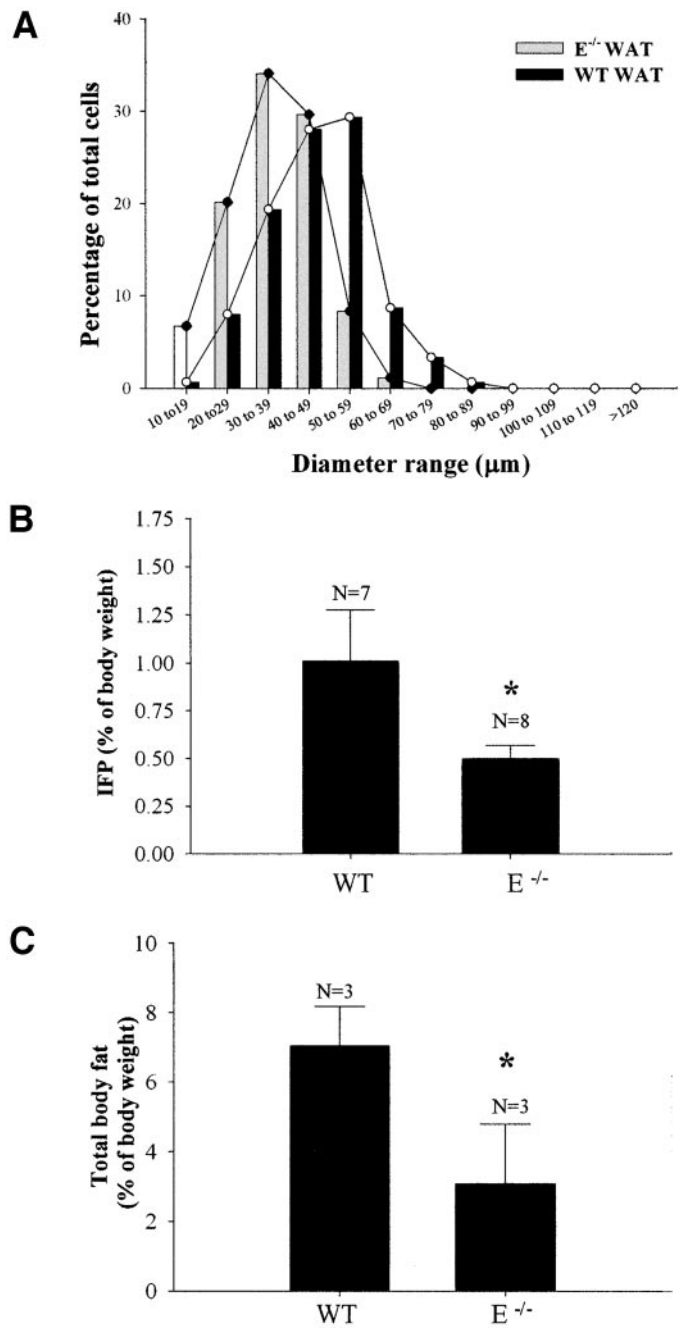

FIG. 1. Adipocyte diameter and adipose tissue mass in apoE$^{-/-}$ mice. White adipose tissue (WAT) was harvested from 10-week-old female apoE$^{-/-}$ (E$^{-/-}$) or wild-type (WT) mice. *A*: Adipocyte size was determined as described in RESEARCH DESIGN AND METHODS. A histogram of size distribution (representative of three separate comparisons) is shown. *B*: IFP weight as a percentage of total body weight is shown. *C*: Total body fat mass was measured by dexa scanning (as described in RESEARCH DESIGN AND METHODS) is presented as a percentage of total body weight. Values shown are means ± SD of triplicate samples. *P < 0.05.

46 ± 16% decrease in the number of lipid-containing cells (as determined by oil red O staining) per well in six separate comparisons (not shown).

To further validate the role of apoE expression in adipocyte triglyceride and free fatty acid content, we used an adenovirus to produce apoE expression in apoE$^{-/-}$ adipocytes. ApoE expression was confirmed by Western blot of adipocyte lysates. In Fig. 3*A* and *B* (*left panels*), wild-type adipocytes have higher triglyceride and free fatty acid levels compared with apoE$^{-/-}$ adipocytes as expected based on results in Fig. 2. Incubation with a LacZ adenovirus produced no change in triglyceride or free fatty acid levels in apoE$^{-/-}$ adipocytes compared with cells incubated without adenovirus. Adenoviral-mediated apoE expression for only 48 h, however, led to an approximate doubling in triglyceride and free fatty acid levels compared with cells incubated with LacZ.

The above results indicated that the absence of apoE expression in adipocytes leads to reduced adipocyte triglyceride mass. To evaluate pathways leading to alteration in triglyceride mass, we next measured triglyceride synthesis and hydrolysis in apoE$^{-/-}$ compared with wild-type adipocytes. Figure 4 shows that decreased triglyceride synthesis (Fig. 4*A*) and increased triglyceride hydrolysis (Fig. 4*C*) both contribute to the overall reduction of triglyceride mass in apoE$^{-/-}$ adipocytes. We also measured the synthesis of free cholesterol as a function of apoE expression in adipocytes. We undertook this measurement because in experimental models of obesity, adipocytes with large triglyceride stores have been shown to have increased expression of hydroxymethylglutaryl-CoA reductase (21,22), the rate-limiting enzyme for cholesterol biogenesis. Based on this and on the observations in Figs. 1 and 2, we predicted that the triglyceride-poor adipocytes that result from absence of apoE expression would also manifest lower levels of cholesterol synthesis. The results in Fig. 4*B* confirm lower cholesterol synthesis in apoE$^{-/-}$ adipocytes.

Endogenous adipocyte apoE expression modulates adipocyte gene expression. The regulation of adipocyte lipid metabolism/content by apoE could be expected to produce downstream effects on the expression of adipocyte genes that are involved in lipid metabolism or genes that are responsive to adipocyte lipid flux/content. The results in Table 2 show the expression level of genes involved in fatty acid transport/oxidation or lipid metabolism and markers of adipocyte differentiation. Gene expression was evaluated in freshly isolated adipocytes and cultured adipocytes from wild-type and apoE$^{-/-}$ mice. Compared with wild-type, apoE$^{-/-}$-cultured adipocytes demonstrated a significant increase in the expression of genes involved in fatty acid transport or oxidation and a significant decrease in markers of adipocyte differentiation. Similar changes were measured in mature adipocytes freshly isolated from adipose tissue. Perilipin mRNA levels were not influenced by the absence of apoE expression, but caveolin mRNAs were substantially reduced in apoE$^{-/-}$ compared with wild-type adipocytes.

Endogenous adipocyte apoE has an important effector role in the adipocyte response to PPAR-γ agonists. Adipocytes respond to PPAR-γ agonists with triglyceride accumulation. We have recently shown that PPAR-γ agonists increase adipocyte and adipose tissue apoE expression in vitro and in vivo (16). In the current study, we show that adipocyte apoE expression leads to increased adipocyte triglyceride content. We, therefore, performed experiments to test the hypothesis that the increased apoE expression after PPAR-γ stimulation was an important functional component of the adipocyte triglyceride accumulation that occurs in response to PPAR-γ stimulation. Addition of rosiglitazone to adipocyte culture over post-induction day (PID) 10–16 produced a fourfold increase in apoE expression in wild-type control cells as measured by Western blotting (not shown). Figure 5 shows the effect of the incubation with rosiglitazone in

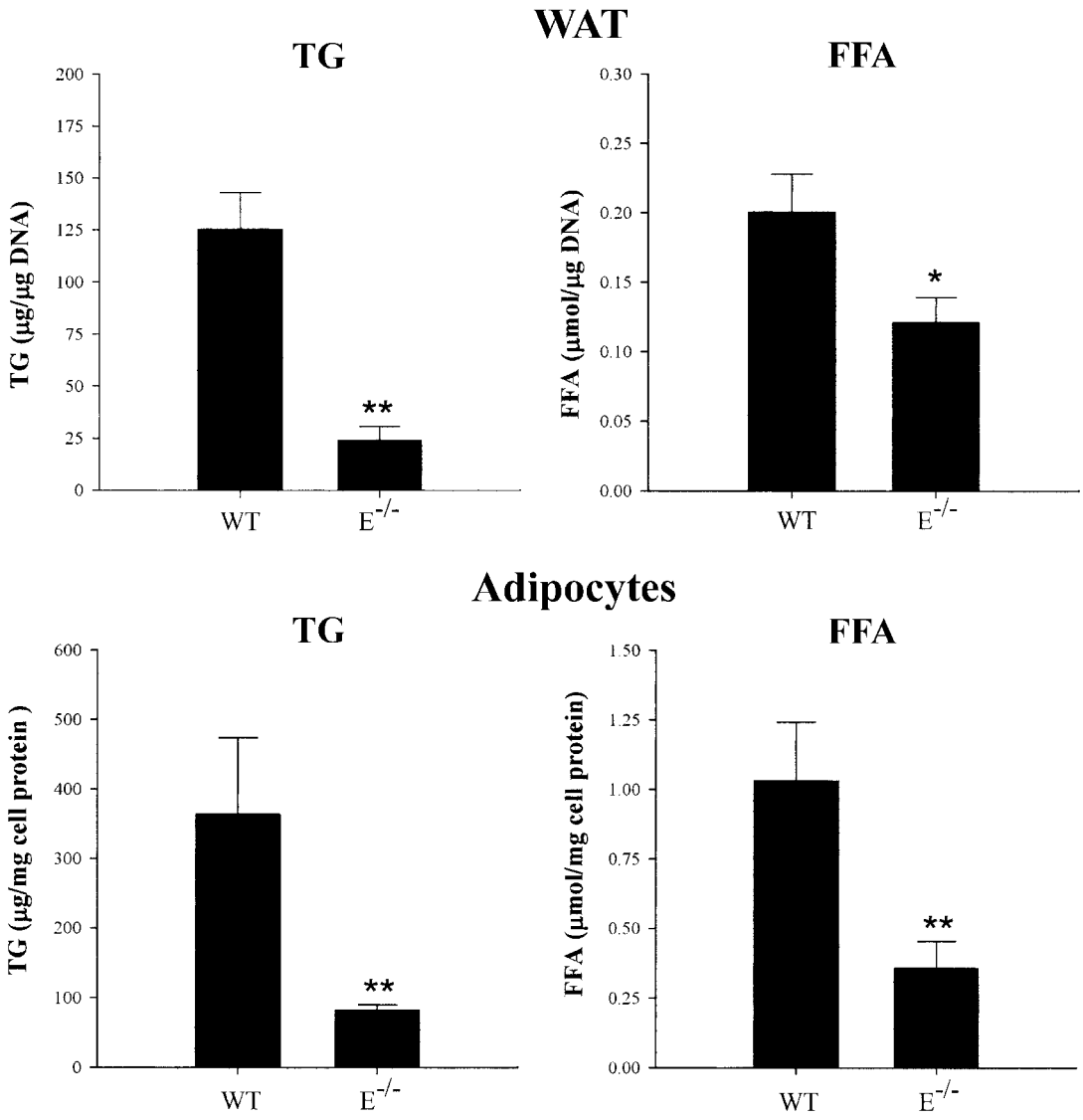

FIG. 2. Triglyceride (TG) and free fatty acid (FFA) mass in adipose tissue and primary adipocytes. Freshly isolated white adipose tissue (WAT) or adipocytes at PID 12 were used for measurement of triglyceride, free fatty acid, DNA, or protein as described in RESEARCH DESIGN AND METHODS. The values shown for white adipose tissue represent the mean ± SD of quadruplicate samples. Values shown for adipocytes represent the means ± SD of triplicate samples. *$P < 0.05$, **$P < 0.01$.

apoE$^{-/-}$ compared with wild-type adipocytes on triglyceride mass (Fig. 5*A*), triglyceride synthesis (Fig. 5*B*), triglyceride hydrolysis (Fig. 5*C*), free cholesterol mass (Fig. 5*D*), and adipocyte perilipin mRNA levels (Fig. 5*E*). In the absence of adipocyte apoE expression, the PPAR-γ–induced increase in triglyceride mass, triglyceride synthesis, and free cholesterol mass is suppressed or abolished. The suppression of triglyceride hydrolysis after rosiglitazone treatment remains intact but does not reach statistical significance in apoE$^{-/-}$ compared with wild-type control adipocytes. Figure 5*E* presents the fold change in perilipin gene expression produced by incubation with rosiglitazone for apoE$^{-/-}$ and wild-type adipocytes. The rosiglitazone-mediated increase in perilipin, fatty acid synthase, CCAAT/enhancer binding protein (CEBP) α, and adiponectin were all significantly blunted in apoE$^{-/-}$ cells.

Adipocyte triglyceride accumulation from VLDL is abolished in the absence of endogenous apoE expression. There are multiple potential pathways by which endogenous apoE could regulate adipocyte triglyceride turnover. An important source of adipocyte triglyceride derives from its interaction with TGRLs. We, therefore, next evaluated the importance of endogenous adipocyte apoE for modulating the triglyceride accumulation that occurs in response to incubation with TGRLs. We incubated freshly harvested adipose tissue from apoE$^{-/-}$ and wild-type control mice with apoE-containing TGRLs for 48 h and evaluated the impact of this incubation on adipose tissue triglyceride content. Figure 6 shows that incubation of wild-type adipose tissue with apoE-containing TGRLs led to a significant increase in adipose tissue triglyceride content compared with the incubation without TGRLs. On the other hand, the incubation of apoE$^{-/-}$ adipose tissue with the same apoE-containing TGRLs did not increase adipose tissue triglyceride content; in fact, adipose tissue triglyceride content slightly fell compared with the incubation without TGRLs. Similar results were obtained after 24-h incubations. These results indicated that endogenous apoE was required for the increase in adipose tissue triglyceride mass that occurs in response to an incubation with TGRLs and further indicate that apoE present in TGRLs cannot substitute for the effect of

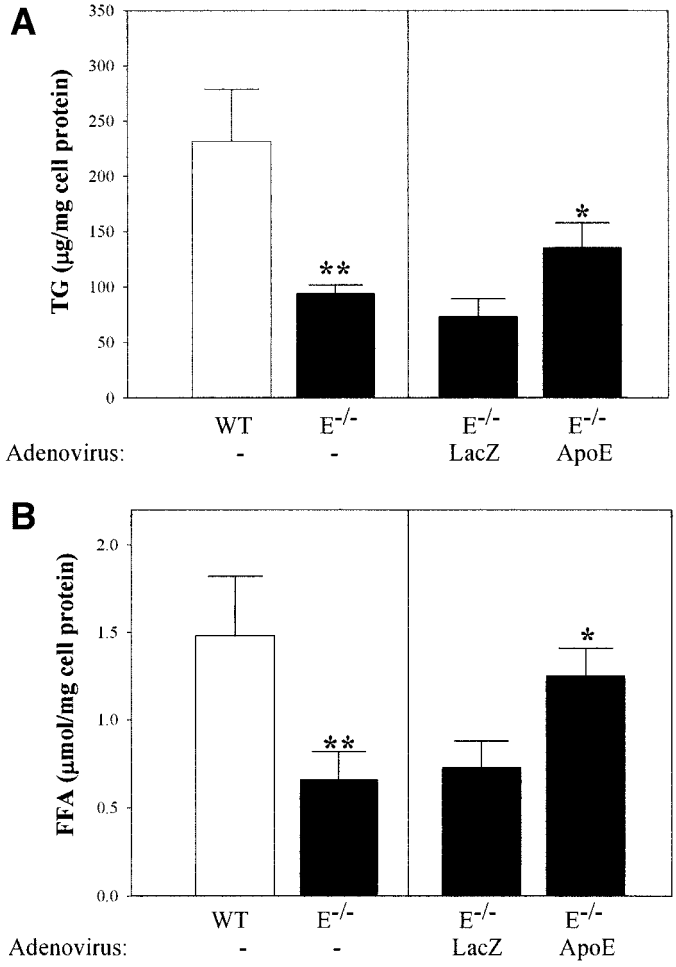

FIG. 3. Effect of apoE expression on triglyceride (TG) and free fatty acid (FFA) mass in apoE$^{-/-}$ (E$^{-/-}$) adipocytes. On PID 10, apoE$^{-/-}$ adipocytes were incubated with a LacZ- or apoE-expressing adenovirus (*right panels* in *A* and *B*) as described in RESEARCH DESIGN AND METHODS. After 48 h, these adipocytes, along with wild-type (WT) adipocytes (□) and apoE$^{-/-}$ adipocytes (■) (*left panels* in *A* and *B*), were harvested for measurement of triglyceride (*A*) and free fatty acid (*B*) mass as described in RESEARCH DESIGN AND METHODS. The values shown are the means ± SD from triplicate samples. *$P < 0.05$, **$P < 0.01$.

endogenous adipocyte apoE to facilitate triglyceride accumulation.

DISCUSSION

ApoE is highly expressed in adipocytes and adipose tissue (7,16). In the current studies, we have established a novel role for endogenously expressed apoE in modulating adipocyte lipid metabolism. ApoE$^{-/-}$ mice on a chow diet have smaller adipocytes and less adipose tissue than wild-type controls (Fig. 1). Importantly, the lower adipose tissue triglyceride and free fatty acid mass observed in freshly isolated adipose tissue from apoE knockout mice is preserved in isolated adipocytes that are derived from preadipocytes and evaluated at PID 12 (Fig. 2). This concordance between freshly isolated adipose tissue and isolated primary adipocytes underlines a physiologic role for endogenous adipocyte apoE in vivo for modulating adipocyte triglyceride and fatty acid content. The direct association between apoE expression and triglyceride and free fatty acid mass in adipocytes is further confirmed by the observation that adenoviral-mediated expression of apoE in apoE$^{-/-}$ adipocytes significantly increases triglyceride and free fatty acid mass in primary adipocytes after 48 h (Fig. 3). The lower triglyceride content in apoE$^{-/-}$ adipocytes results from both a decrease in triglyceride synthesis and an increase in triglyceride hydrolysis (Fig. 4).

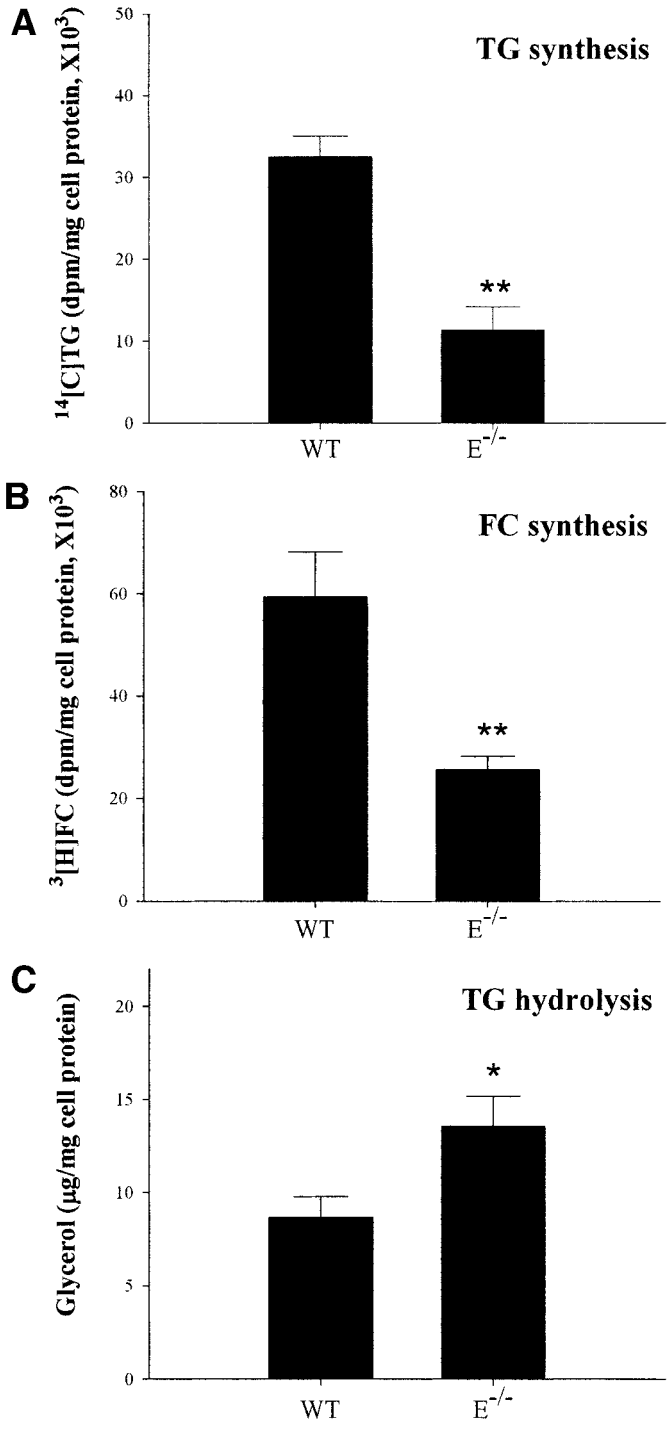

FIG. 4. Effect of apoE expression on triglyceride (TG) synthesis (*A*), free cholesterol (FC) synthesis (*B*), and triglyceride hydrolysis (*C*) in adipocytes. Adipocytes at PID 10 were pulse labeled with 0.25 μCi/ml [^{14}C]oleate (*A*) or 50 μCi/ml [^{3}H]acetate (*B*) for 2 or 4 h, respectively, to measure triglyceride or free cholesterol synthesis as described in RESEARCH DESIGN AND METHODS. After incubation, cells were washed and lipids extracted and separated by thin-layer chromatography. *C*: Triglyceride hydrolysis was determined by measuring release of free glycerol into the medium over 2 h as described in RESEARCH DESIGN AND METHODS. Values shown are means ± SD from triplicate samples. *$P < 0.05$, **$P < 0.01$.

TABLE 2
Gene expression in apoE$^{-/-}$ compared with wild type from 10-day or freshly isolated adipocytes

	ApoE$^{-/-}$/WT	
	Cultured adipocytes	Freshly isolated adipocytes
Fatty acid oxidation		
ACADM	1.6 ± 0.10*	8.3 ± 0.7†
PGC-1α	9.7 ± 0.80†	7.9 ± 1.0†
CPT-1	1.5 ± 0.1*	2.3 ± 0.2†
ACO	4.3 ± 0.4*	
Adipocyte differentiation		
Adiponectin	0.2 ± 0.01†	0.2 ± 0.0†
CEBPα	0.4 ± 0.2*	
PPAR-γ	0.5 ± 0.2*	0.4 ± 0.1†
Lipid trafficking		
Perilipin	1.2 ± 0.20	
Caveolin-1	0.1 ± 0.01†	

Total RNA was isolated from adipocytes at day 10 in culture or from freshly isolated adipocytes from adipose tissue digests. Levels of mRNA for indicated targets were quantitated as described in RESEARCH DESIGN AND METHODS. Results are expressed as fold change in mRNA level in apoE$^{-/-}$ compared with wild-type adipocytes and represent results from nine separate cell preparations each done in triplicate. *$P < 0.05$, †$P < 0.01$. ACADM, acetyl-CoA dehydrogenase, medium chain; ACO, acyl-CoA oxidase; CPT-1, carnitine palmitoyltransferase-1; PGC-1, PPAR-γ coactivator.

Several potential mechanisms can be considered for the effect of apoE on adipocyte lipid metabolism. One mechanism that appears to be involved is the accumulation of triglyceride that occurs in response to interaction with TGRLs (Fig. 6). Others have reported that exogenous apoE in VLDL is important for adipocyte triglyceride accumulation (23), but the result in Fig. 6 clearly demonstrates that apoE in TGRLs, which induces VLDL, cannot substitute for the effect of endogenous apoE on triglyceride accumulation. The result in Fig. 6 also raises a number of interesting questions for future investigation. The increase in triglyceride in wild-type but not apoE$^{-/-}$ tissue could be related to differences in holoparticle uptake, differences in the use of fatty acids in the TGRL triglyceride or phospholipid, or differences in TGRL-dependent lipid signaling that influence the adipocyte utilization of fatty acids or fatty acid precursors present in serum. For example, components of TGRL could activate PPAR-γ or PPAR-δ receptors in adipocytes (17,24) in an apoE-dependent manner. In other model systems, endogenous cellular apoE has been shown to influence interaction of lipoproteins with cell surface receptors or proteoglycans (25,26). Endogenous expression of apoE in macrophages, for example, forms a proteoglycan-bound cell surface layer that appears to act as a bridge to anchor lipoproteins at the cell surface (26,27). A similar effect in adipocytes could anchor TGRL to plasma membrane and thereby facilitate either holoparticle uptake or fatty acid release from TGRL core triglyceride.

Beyond modulating triglyceride accumulation subsequent to TGRL interaction, other mechanisms by which endogenous apoE could influence adipocyte triglyceride content and turnover must also be considered. ApoE has a well-established role in other cell types for modulating cellular sterol metabolism and flux, and sterol and triglyceride storage are tightly linked in adipocytes (8–15,22). ApoE modulation of adipocyte cholesterol content and/or distribution could influence plasma membrane structure, including the function and/or number of caveolae. These plasma membrane subdomains have been shown to be important for facilitated fatty acid transport across adipocyte plasma membrane, and a subset of these have been shown to synthesize triglyceride (28,29). Free cholesterol is also an important structural component of the triglyceride droplet where it accumulates at the cytoplasmic interface. Finally, in steroidogenic cells, apoE expression has been shown to modulate second messenger signaling pathways (30). This represents another potential mechanism for apoE effects on triglyceride turnover in adipocytes.

Our results show that the absence of apoE expression in adipocytes also influences adipocyte gene expression. The decreased expression of differentiation markers with apoE deficiency was noted in freshly isolated adipocytes and in adipocytes differentiated from preadipocytes in culture. Freshly isolated adipocytes from apoE$^{-/-}$ mice are smaller, indicating less triglyceride per cell, and cultured adipocytes from apoE$^{-/-}$ mice have lower level of triglyceride per milligram of protein. Cultures of adipocytes from apoE$^{-/-}$ mice also contain fewer lipid-containing cells, but whether there is also less lipid per lipid-containing cell is not addressed by our data. All of these results are consistent with a defect in adipocyte differentiation resulting from absent endogenous adipocyte apoE expression, which is then reflected in altered adipocyte lipid metabolism. However, a somewhat more provocative alternative is that apoE deficiency produces a primary defect in lipid flux, which then impacts adipocyte differentiation. For example, caveolin expression can be modulated by cellular free cholesterol (31), and changes in adipocyte cholesterol flux mediated by apoE could lead to downstream changes in caveolin expression. Changes in caveolin expression have been shown to have important implications for triglyceride droplet biogenesis and metabolism (32). Furthermore, lipid metabolites serve as ligands for PPAR-γ and PPAR-δ receptors, which are involved in adipocyte function and differentiation (17,24).

One of the genes with reduced expression in apoE$^{-/-}$ adipocytes was adiponectin. This is different than what might be expected based on observations that leanness and small adipocytes are associated with increased adiponectin expression (33,34). However, changes in adipocyte size resulting from leanness may produce different effects on adiponectin expression than changes in size resulting from absent apoE expression. ApoE$^{-/-}$ adipocytes also have increased expression of genes related to fatty acid metabolism; for example, there were substantial increases in PGC-1α expression. PGC-1α is involved in mitochondrial biogenesis and, in vivo, has been shown to be highly responsive to physiologic conditions that require increased mitochondrial energy production (35,36). Interestingly, we measured lower cellular fatty acid levels in apoE$^{-/-}$ adipocytes even though they have increased release of free glycerol suggesting increased release of fatty acids from triglyceride. The fall in cellular fatty acid level in the absence of apoE expression therefore most likely results from either decreased uptake of fatty acids or the increased oxidation of fatty acids; as suggested by changes in ACADM-1 and PGC-1α expression.

One of the most important observations in this manuscript is the role played by apoE in the lipogenic response to PPAR-γ agonists in adipocytes. Treatment of adipocytes or whole animals with PPAR-γ agonists leads to an increase in adipocyte triglyceride mass (17,37). Treatment of isolated adipocytes or whole animals with PPAR-γ agonists also leads to an increase of apoE expression in

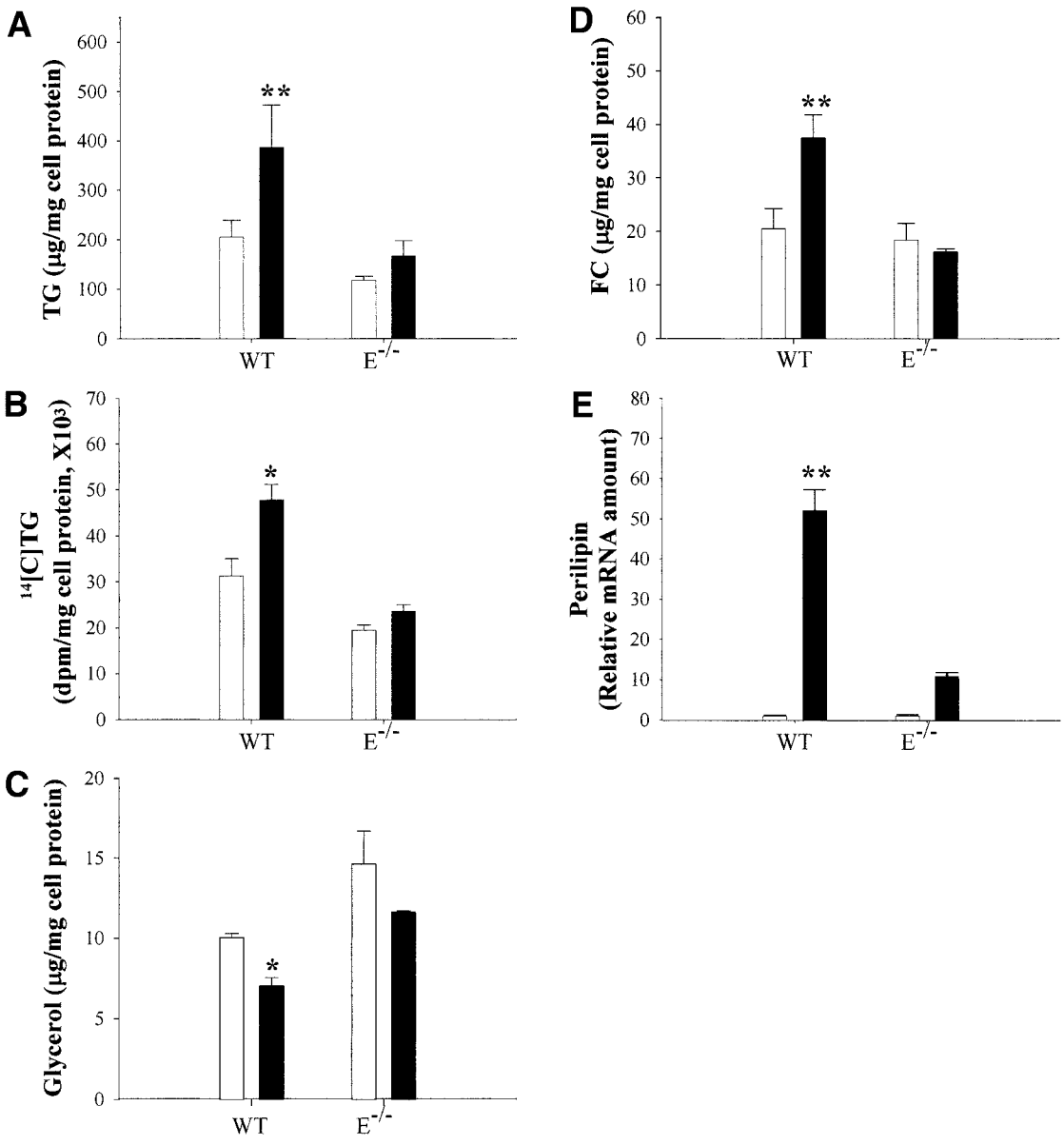

FIG. 5. Role of apoE in mediating PPAR-γ effects in adipocytes. Adipocytes isolated from apoE$^{-/-}$ or wild-type mice at PID 10 were incubated without or with 5 μmol rosiglitazone for 6 days in 10% FBS/DMEM. At that time, cells were harvested for measurement of triglyceride mass (*A*), triglyceride synthesis (*B*), triglyceride hydrolysis (*C*), free cholesterol mass (*D*), or perilipin gene expression (*E*), all as described in RESEARCH DESIGN AND METHODS. *A–D*: Values shown represent the means ± SD of triplicate samples. □, control; ■, rosiglitazone. *$P < 0.05$, **$P < 0.01$ for differences between cells incubated with or without rosiglitazone. *E*: Values are from nine separate cell preparations each done in triplicate. *$P < 0.5$, **$P < 0.01$ comparing fold increase with rosiglitazone in wild-type compared with apoE$^{-/-}$ cells.

adipose tissue and adipocytes (16). In this study, we have shown that the adipocyte triglyceride accumulation that occurs in response to PPAR-γ agonists is largely absent in the absence of adipocyte apoE expression. Therefore, the increased apoE expression in response to PPAR-γ agonists likely plays a key role for triglyceride accumulation in adipocytes subsequent to PPAR-γ stimulation.

The novel findings in this manuscript, that endogenous apoE modulates adipocyte lipid metabolism and is important for the effect of PPAR-γ agonists on adipocyte lipid metabolism, raise important physiologic issues for consideration. One important question relates to physiologic factors that regulate adipocyte apoE expression. We have previously shown that treatment of humans with PPAR-γ agonist results in increased apoE mRNA levels in adipose tissue (16). However, regulation of adipocyte apoE expression may be complex and may also respond to changes in systemic hormone levels, substrate flux, or cytokines produced by adipose tissue macrophages. There could also be species-dependent differences related to the cell type in adipose tissue that expresses apoE. For example, Blaner and colleagues (38) have reported that in rat adipose tissue, most apoE expression is localized to the stromal-vascular fraction. Furthermore, dysregulation of adipocyte apoE expression could occur in states of chronic obesity or insulin resistance. Additional physiologic regulators of adipocyte apoE expression in vivo remain to be defined. In human obesity, important differences have been described for adipose tissue present in subcutaneous compared with visceral depots (39,40). It will be of interest to determine whether the regulation of apoE expression or the effect of apoE expression on adipocyte lipid metabolism differs between these two sites. In conclusion, the results in this report support the

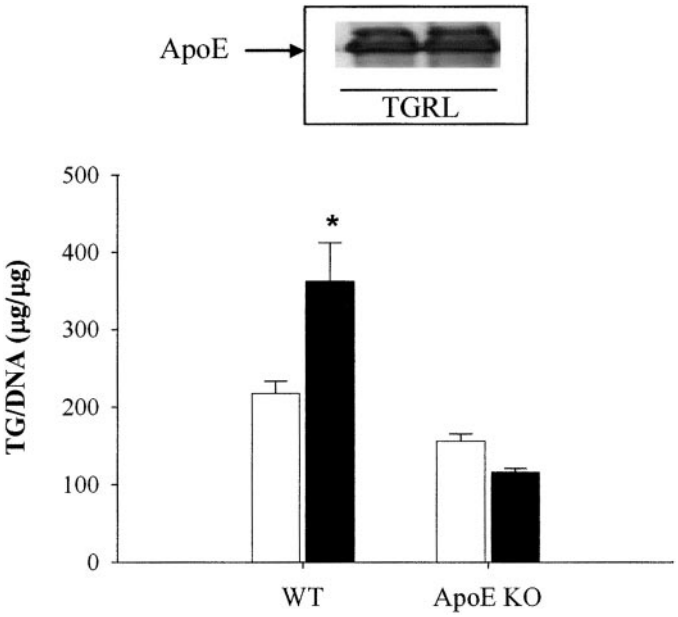

FIG. 6. Change in adipose tissue triglyceride content after incubation with apoE-containing TGRL. Freshly harvested adipose tissue from apoE$^{-/-}$ or wild-type (WT) mice was incubated with or without 100 μg/ml apoE-containing TGRLs for 48 h in 1% lipoprotein-deficient FBS. After this incubation, triglyceride and DNA levels were measured as described in RESEARCH DESIGN AND METHODS. Values shown are means ± SD of triplicate samples. Before the incubation, triglyceride content was 196 ± 30 and 91 ± 12 μg/mg for wild-type and apoE$^{-/-}$ adipose tissue, respectively. *$P < 0.05$ for the comparison between incubations with and without TGRL in each genotype. □, no addition; ■, TGRL. *Top*, ApoE content of TGRL confirmed by Western blot.

hypothesis that endogenous adipocyte apoE expression is an important modulator of adipocyte lipid metabolism, influencing adipocyte triglyceride mass and synthesis, free fatty acid mass, cholesterol synthesis, and the expression of genes involved in triglyceride droplet metabolism and fatty acid oxidation. They also provide a basis for new areas of investigation aimed at integrating adipocyte apoE into a comprehensive model of adipose tissue physiology.

ACKNOWLEDGMENTS

T.M. has received a gift from the Svendsen Family Foundation. This work was supported by National Institutes of Health Grant DK-71711.

We thank Dr. Giamila Fantuzzi for assistance with the dexa measurements and Stephanie Thompson for assistance with manuscript preparation.

REFERENCES

1. Lakka H, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomlehto J, Salonen JT: The metabolic syndrome and total cardiovascular disease mortality in middle-aged men. *JAMA* 288:2709–2716, 2002
2. Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC: Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 25:1129–1134, 2002
3. Ginsberg HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453–458, 2000
4. Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP: Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation* 101:975–980, 2000
5. Fruhbeck G, Gomez-Ambrosi J, Muruzabal J, Muruzabal FJ, Burrell MA: The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J Physiol Endocrinol Metab* 280:E827–E847, 2001
6. Kershaw EE, Flier JS: Adipose tissue as an endocrine organ. *J Clin Endocrin Metab* 89:2548–2556, 2004
7. Zechner R, Moser R, Newman TC, Fried SK, Breslow JL: Apolipoprotein E gene expression in mouse 3T3–L1 adipocytes and human adipose tissue and its regulation by differentiation. *J Biol Chem* 266:10583–10588, 1991
8. Basu SK, Ho YK, Brown MS, Bilheimer DW, Anderson RGW, Goldstein J: Biochemical and genetic studies of the apoprotein E secreted by mouse macrophages and human monocytes. *J Biol Chem* 257:9788–9795, 1982
9. Mazzone T, Gump H, Diller P, Getz GS: Macrophage free cholesterol content regulates apolipoprotein E synthesis. *J Biol Chem* 262:11657–11662, 1987
10. Mazzone T, Basheeruddin K, Poulos C: Regulation of macrophage apolipoprotein E gene expression by cholesterol. *J Lipid Res* 30:1055–1064, 1989
11. Wyne KL, Schreiber JR, Larsen AL, Getz GS: Regulation of apolipoprotein E biosynthesis by cAMP and phorbol ester in rat ovarian granulosa cells. *J Biol Chem* 264:981–989, 1989
12. Mazzone T, Reardon C: Expression of heterologous human apolipoprotein E by J774 macrophages enhances cholesterol efflux to HDL_3. *J Lipid Res* 35:1345–1353, 1994
13. Lin C-Y, Duan H, Mazzone T: Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic analysis and divergent mechanism for endogenous versus exogenous apolipoprotein E. *J Lipid Res* 40:1618–1626, 1999
14. Prack MM, Rothblat GH, Erikson SK, Reyland ME, Williams DL: Apolipoprotein E expression in Y1 adrenal cells is associated with increased intracellular cholesterol content and reduced free cholesterol efflux. *Biochemistry* 33:5049–5055, 1994
15. Reyland ME, Gwynne JT, Forgez P, Prack MM, Williams DL: Expression of the human apolipoprotein E gene suppresses steroidogenesis in mouse Y1 adrenal cells. *Proc Natl Acad Sci U S A* 88:2375–2379, 1991
16. Yue L, Rasouli N, Ranganathan G, Kern PA, Mazzone T: Divergent effects of PPARγ agonists and TNFα on adipocyte apoE expression. *J Biol Chem* 279:47626–47632, 2004
17. Lowell BB: PPARγ: an essential regulator of adipogenesis and modulator of fat cell function. *Cell* 99:239–242, 1999
18. Bogacka I, Xie H, Bray GA, Smith SR: Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. *Diabetes* 54:1392–1399, 2005
19. Ruan H, Miles PDG, Ladd CM, Ross K, Golub TR, Olefsky JM, Lodish HF: Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-α: implications for insulin resistance. *Diabetes* 51:3176–3188, 2002
20. Mazzone T, Lopez C, Bergstraesser L: Modification of very low density lipoprotein leads to macrophage scavenger receptor uptake and cholesteryl ester deposition. *Arteriosclerosis* 7:191–196, 1987
21. Soukas A, Cohen P, Socci ND, Friedman JM: Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 14:963–980, 2000
22. Le Lay S, Krief S, Farnier C, Lefrere I, Le Liepvre X, Bazin R, Ferre P, Dugail I: Cholesterol: a cell size dependent signal which regulates glucose metabolism and gene expression in adipocytes. *J Biol Chem* 276:16904–16910, 2001
23. Chiba T, Nakazawa T, Yui K, Kaneko E, Shimokado K: VLDL induces adipocyte differentiation in apoE-dependent manner. *Arterioscler Thromb Vasc Biol* 23:1423–1429, 2003
24. Chawla A, Lee C-H, Barak Y, He W, Rosenfeld J, Liao D, Han J, Kang H, Evans RM: PPARδ is a very low-density lipoprotein sensor in macrophages. *Proc Natl Acad Sci U S A* 100:1268–1273, 2003
25. Vassiliou G, McPherson R: A novel efflux-recapture process underlies the mechanism of high-density lipoprotein cholesteryl ester-selective uptake mediated by the low-density lipoprotein receptor-related protein. *Arterioscler Thromb Vasc Biol* 24:1669–1675, 2004
26. Lin C-Y, Lucas M, Mazzone T: Endogenous apoE expression modulates HDL3 binding to macrophages. *J Lipid Res* 39:293–301, 1998
27. Lucas M, Mazzone T: Cell-surface proteoglycans modulate net synthesis and secretion of macrophage apoE. *J Biol Chem* 271:13454–13460, 1996
28. Pohl J, Ring A, Ehehalt R, Schulze-Bergkamen H, Schad A, Verkade P, Stremmel W: Long-chain fatty acid uptake into adipocytes depends on lipid raft function. *Biochemistry* 43:4179–4187, 2004
29. Ost A, Ortegren U, Gustavsson J, Nystrom FH, Stralfors P: Triacylglycerol is synthesized in a specific subclass of caveolae in primary adipocytes. *J Biol Chem* 280:5–8, 2005
30. Reyland ME, Williams DL: Suppression of cAMP-mediated signal transduction in mouse adrenocortical cells which express apolipoprotein E. *J Biol Chem* 266:21099–21104, 1991
31. Hailstones D, Sleer LS, Parton RG, Stanley KK: Regulation of caveolin and caveolae by cholesterol in MDCK cells. *J Lipid Res* 39:369–379, 1998
32. Cohen AW, Razani B, Schubert W, Williams TM, Wang XB, Iyengar P,

Brasaemle DL, Scherer PE, Lisanti MP: Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. *Diabetes* 53:1261–1270, 2004
33. Yildiz BO, Suchard MA, Wong ML, McCann SM, Licinio J: Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A* 101:10434–10439, 2004
34. Berg AH, Scherer PE: Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 96:939–949, 2005
35. Finck BN, Kelly DP: PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 116:615–622, 2006
36. Orci L, Cook WS, Ravazzola M, Wang M-Y, Park B-H, Montesano R, Unger RH: Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc Natl Acad Sci U S A* 101:2058–2063, 2004
37. Ferre P: The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes* 53 (Suppl. 1):S43–S50, 2004
38. Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS, Blaner WS: Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 267:1805–1810, 1992
39. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW: Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 145:2273–2282, 2004
40. Fried SK, Russell CD, Grauso NL, Brolin RE: Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J Clin Invest* 92:2191–2198, 1993